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Original article

Deciphering the genetics of antagonism and antimicrobial resistance in *Bacillus velezensis* HU-91 by whole genome analysis

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ABSTRACT

Bacillus species are well reported plant growth promoting rhizobacteria which play an important role in improving soil fertility, nutrient recycling, and controlling phytopathogens. Their use as biocontrol agents is an ecofriendly strategy but being a rhizospheric community, they could harbor antimicrobial resistance genes. In current study, rhizobacteria were isolated from the mono cropped wheat. Ten out of 50 isolates showed antifungal activity against *Fusarium* spp., economically important pathogens of wheat. The strain HU-91 with highest antifungal activity showed the biochemical traits of lipopeptide production. It also resisted multiple antibiotics of different classes (index $\geq$ 0.2) and was categorized as multidrug resistant bacteria. The strain HU-91 was identified as *Bacillus velezensis* based on its 16S rRNA homology (99 %). Whole genome analysis of *B. velezensis* HU-91 revealed 76 contigs with a total length of 3,952,697 bp, GC content 46.3 %, and a total of 3912 genes. Functional classification of genome predicted genes involved in drug resistance, and antagonism. A detailed genome analysis identified CAZymes and 10 biosynthetic gene clusters involved in antagonism. Various antimicrobial resistance genes associated with the resistance to different antimicrobial classes such as β lactams, tetracyclines, aminoglycosides, fluoroquinolones, macrolides, sulfonamides, and lincosamides were also found in the genome of HU-91. Furthermore, HU-91 lacked plasmid but harbored a well-developed CRISPR Cas system. The underlying mechanisms involved in the antimicrobial resistance were depicted as intrinsic based on the genes encoding production of antibiotics degrading enzymes and efflux pumps. These findings depict that HU-91 adapts the intrinsic mechanisms of drug resistivity to tolerate environmental stress. The lack of plasmid and presence of CRISPR Cas system advocate its safety to be used as bioinoculant.

1. Introduction

Phytopathogens, that cause economically important diseases are a widely reported problem in agriculture as they pose deleterious effects on crops (Anand et al., 2023). *Fusarium* spp. are the most devastating phytopathogen for cereal crops especially wheat grown in arid and semi-arid regions where wheat is mono cropped to meet the needs of growing population (Ullah et al., 2020). Different strategies have been employed to control the deleterious effects of pathogens on crops.

Use of biocontrol agents (*Bacillus* spp., *Pseudomonas* spp., etc.) is an interesting recourse to inhibit the growth of phytopathogens. Biocontrol agents adapt different strategies to cope with biotic and abiotic stresses such as production of antibiotics, siderophores, cell wall degrading enzymes, and volatile organic compounds (Zhang et al., 2021). These secondary metabolites not only provide interference competition but usually contribute to the self-resistance against the antimicrobial metabolites especially antibiotics (Xia et al., 2022). This self-protection of antibiotic producers is referred to as innate resistance while the appearance of self-protection in non-antibiotic producers is referred to as acquired resistance. Both types of bacteria, the producers, and non-producers of antibiotics, can achieve innate as well as acquired resistance. Thus, the secondary metabolites producing microbes have been an extensive source of antibiotic resistance genes (Wencewicz, 2019).

The antibiotic resistance genes of bacteria could transfer to other

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Table 1
Morphological characteristics and antagonistic activity of rhizobacteria.

Isolate code	Endosphere/Rhizosphere	Colony morphology		[1]Gram reaction	[2]Antagonistic activity (%)			
		Shape	Margin		*F. oxysporum*	*F. moniliforme*	*F. graminearum*	*F. solani*
HU-91	Endosphere/Rhizosphere	Round	Irregular	+	$51.66^{a} \pm 0.65$	$52.85^{a} \pm 0.71$	$48.51^{a} \pm 1.18$	$55.93^{a} \pm 0.91$
HU-92	Endosphere	Oval	Smooth	+	$33.80^{d} \pm 0.72$	$48.59^{b} \pm 0.83$	–	–
HU-93	Rhizosphere	Round	Irregular	+	$29.93^{e} \pm 0.50$	–	$25.88^{e} \pm 0.83$	$25.21^{e} \pm 0.93$
HU-94	Rhizosphere	Round	Irregular	+	$25.59^{f} \pm 0.81$	$36.99^{d} \pm 0.40$	–	$52.89^{b} \pm 0.72$
HU-95	Endosphere/Rhizosphere	Round	Irregular	+	$21.74^{g} \pm 0.84$	$30.22^{e} \pm 0.60$	$21.85^{f} \pm 0.81$	$20.40^{f} \pm 0.74$
HU-96	Rhizosphere	Oval	Irregular	–	$45.63^{b} \pm 0.72$	$32.77^{d} \pm 0.73$	–	–
HU-97	Endosphere	Round	Irregular	–	$37.25^{c} \pm 0.82$	$41.11^{c} \pm 0.56$	$28.55^{de} \pm 0.56$	$33.22^{c} \pm 0.77$
HU-98	Rhizosphere	Oval	Smooth	–	–	$26.83^{f} \pm 0.60$	$36.83^{c} \pm 0.60$	–
HU-99	Rhizosphere	Round	Irregular	+	–	$16.92^{g} \pm 0.83$	$31.15^{d} \pm 0.55$	$28.07^{d} \pm 0.59$
HU-100	Rhizosphere	Round	Irregular	+	–	–	$42.70^{b} \pm 1.08$	–

[1] + (Gram positive) - (Gram negative).
[2] - (no antagonism) F (*Fusarium*).

Table 2
Biochemical and genetic profile of lipopeptides in *B. velezensis* HU-91.

Biochemical attributes of lipopeptides	
Biochemical trait	**Activity**
BATH activity	80 % ±3
Oil spread activity	2.12 cm ± 0.56
Emulsification activity	73 % ± 2
Genetics of lipopeptides	
Lipopeptide name	**Genes**
Surfactin	*srfAB, srfAA, srfAC, srfAD*
Fengycin	*fenABCDE*
Bacillolysin	*npr*
Plipastatin	*ppsB-ppsE*

microbes through horizontal gene transfer (HGT) which usually involves mobile genetic elements like transposons, plasmid and integrons. HGT occurs through transformation, transduction, and conjugation (Vinayamohan et al., 2022). Based on resistance pattern, bacteria can be multidrug resistance (MDR), extensive drug resistance (XDR) and pan drug resistance (PDR) (Magiorakos et al., 2012).

The genetic mechanisms adapted by bacteria to resist antimicrobial agents includes inhibition of antibiotics, target modification, enzymatic inactivation of antibiotics, efflux pumps and cell wall permeability. Many of the biocontrol agents such as *Pseudomonas* spp. and *Bacillus* spp. are characterized as MDR (Fahsi et al., 2019).

Besides the natural producers of antibiotics, human anthropogenic activities such as use of pesticides, and antibiotics could also promote antimicrobial resistance. The presence of these compounds provides a selective pressure on dispersal of antimicrobial resistance genes (ARGs) (Mazhar et al., 2021). The use of bioinoculants in agriculture are usually considered as environment friendly but they could be a potential risk for dissemination of ARGs in the environment. It is therefore necessary to ensure the biosafety, efficacy, and quality of the bio products, especially their antimicrobial resistance profile.

Many approaches like amplification of genes, Sanger sequencing, and metagenomics, have been used to detect the genetics, diversity, and evolution of bacteria. However, the genetics of antimicrobial resistivity and antagonism had not been deciphered yet. The next generation

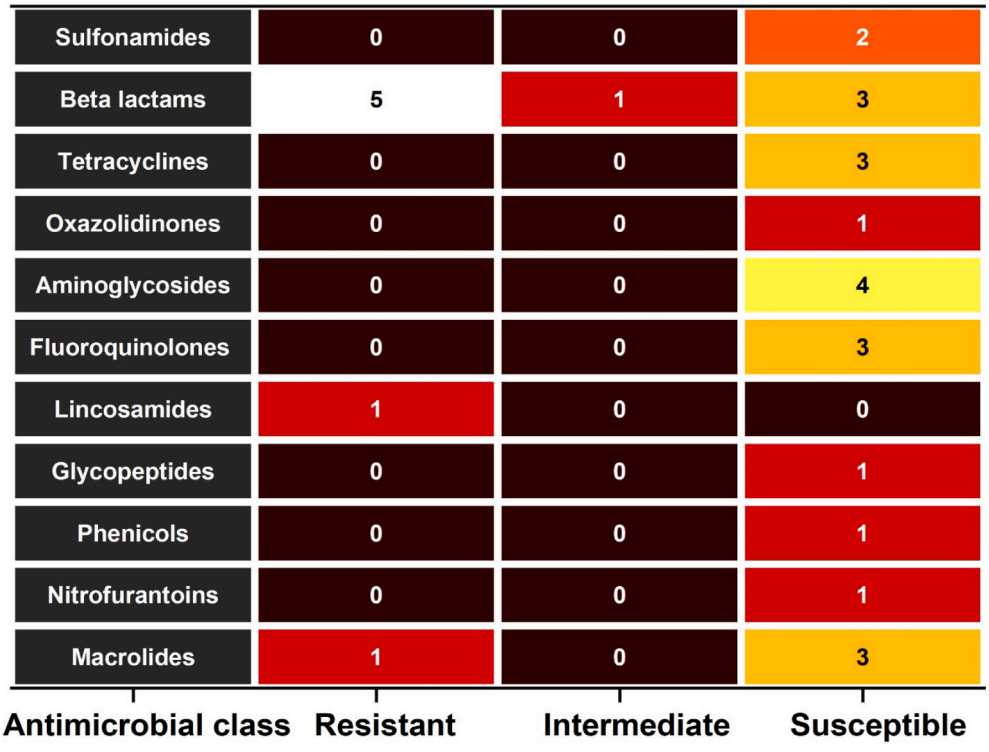

Fig. 1. Heat map indicating the antimicrobial resistance of *B. velezensis* HU-91. Values in the box against each category represent the number of antimicrobial agents.

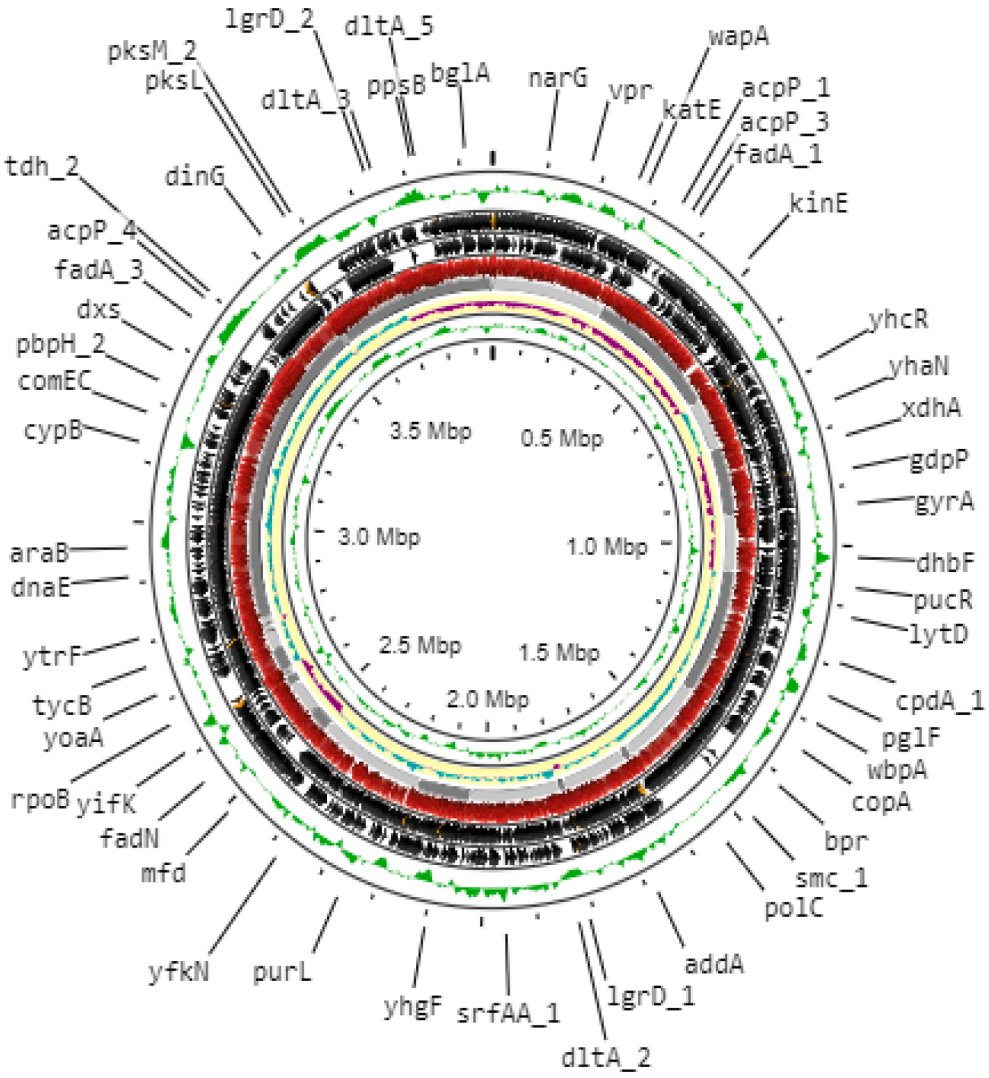

Fig. 2. Circular genome map of *B. velezensis* HU91. From outer to inner circle [tRNA, rRNA and open reading frame (green, black, blue)). Middle circle (red) (Blast sequence similarity with reference genome (*B. velezensis* FZB42)]. Inner circle (GC skew and GC content).

sequencing is a powerful approach for deciphering the underlying genetics of different mechanisms in bacteria through whole genome analysis (WGA). Several studies have mentioned the significance of WGA in prediction of ARGs and antagonism (Wang et al., 2023). WGS has a higher throughput, more flexible and has greater sensitivity over Sanger sequencing. This approach provides new insight into the genetics of unique genes and correlation with their phenotypes (Argimón et al., 2020). The present study aims to isolate bacteria antagonistic to the economically important pathogens (*Fusarium* spp.), from mono cropping system of wheat, elucidate its antimicrobial resistance phenotype and decipher the underlying genetics of its antagonism and antimicrobial resistance by whole genome analysis.

2. Materials and methods

2.1. Field site and sample collection

A mono cropped field under the cultivation of wheat for 6–7 consecutive years was identified in a rainfed area with subtropic semi-arid conditions of District Chakwal, Pakistan (32.8322° N, 72.6151° E). Plants (n = 5) were sampled at the pre-harvesting stage. Healthy plants showing variable height, color, size, and the number of grains in each panicle were uprooted randomly with bulk rhizospheric soil from different places (corner and center with 2 m distance between plants) of the field. Samples were placed in a sterilized polythene zip bag (60 × 90 cm, hole at one end for aeriation), tagged and stored at 4°C for further processing.

2.2. Isolation of bacteria

Bacteria were isolated from rhizosphere and endosphere by serial dilution method. The rhizospheric soil tightly adhering to different plant roots was gently removed, whereas for the endospheric sample, roots were pooled to make a composite sample. Root samples were surface sterilized with 0.1 % mercuric chloride ($HgCl_2$) for 1–2 min and washed 3 times with sterilized distilled water. The plant roots were dried with sterile filter paper and crushed in sterile pestle mortar. A serial dilution ($10^{-1} - 10^{-9}$) was made from 1 g of rhizospheric soil or crushed plant roots and spread on sterile Luria Bertani agar plates. The plates were incubated until the appearance of the colonies (Ullah et al., 2020).

2.3. Antagonistic activity

The selected isolates were tested for their antifungal activity against economically important pathogens such as *F. oxysporum*, *F. graminearum, F. solani* and *F. moniliforme* using a dual culture plate

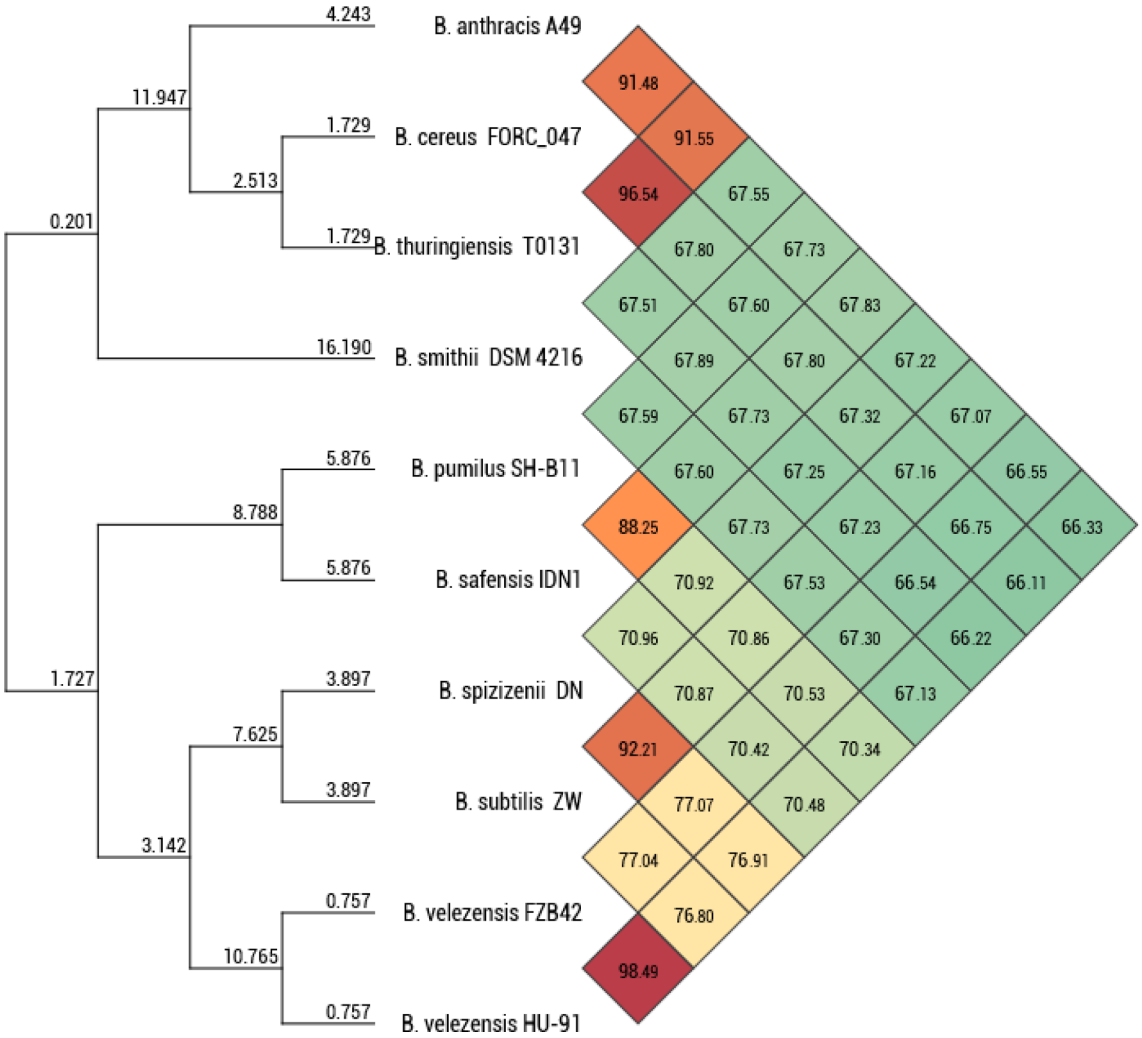

Fig. 3. Phylogenetic tree showing similarity of the *B. velezensis* HU-91 with other *Bacillus* spp. The tree was constructed based on average nucleotide identity (ANI) which was calculated by using Orthologous Average Nucleotide Identity Tool (OAT). Numeric values on the branch represent branch length and values in colored boxes represent percentage sequence similarity.

method. The fungal disc (5 mm) was placed at the center and the bacterial strain was streaked 4 cm away from the fungal disc. The antagonistic activity was observed after 7 days of inoculation and measured using the following formula. *B. velezensis* SM39 (Mulk et al., 2022) was used as a positive control. Cell free broth was used as a negative control.

$$\text{Inhibition}\,\% = \text{C} - \text{T}/\text{C} \times 100\,(\text{C} = \text{mycelium growth under control; T} = \text{mycelium growth uunder treatment})$$

2.4. Biochemical detection of lipopeptides

Lipopeptides were detected in HU-91 by centrifugation (20 min, 10,000 rpm) of overnight grown culture. The supernatant was used for different biochemical analyses. *Bacillus cabrialesii* SM 93 (unpublished data) was used as a positive control.

2.4.1. Hydrophobicity assay (BATH)

The harvested cells were washed twice with a 50 mM potassium phosphate buffer and were re-suspended in the same buffer to optical density 600 nm (0.51). The cell suspension was mixed with tested hydrocarbon (p-xylene), and vortexed (3 min). The mixture was allowed to stand for 45 min and the aqueous phase was subjected to spectrophotometer to measure the OD at 610 nm. Cell hydrophobicity was measured by the following formula (Rosenberg et al., 1980).

$$BATH(\%) = [(B_0 - B)/B_0] \times 100$$

Where B_0 and B = Optical density before and after addition of hydrocarbons respectively.

2.4.2. Oil spread assay

For oil spread assay, 10 µL of bacterial suspension, 20 µL crude oil and 25 mL distilled water was mixed in a petri plate. Appearance of a hallow zone indicated the presence of oil displacement (Zargar et al., 2022).

2.4.3. Emulsification assay

For emulsification assay, cell free supernatant was mixed with crude oil. The suspension was vortexed and incubated at room temperature for 24 h. The E_A was measured by the following formula (Cooper & Goldenberg, 1987).

$$E_A = [\text{Height of the emulsification layer}/\text{Total height of liquid (cm)}] \times 100$$

2.5. Antimicrobial susceptibility testing

The overnight-grown culture of HU-91 was subjected to antimicrobial susceptibility testing by using a disc diffusion assay following Clinical Standards Laboratory Institute (CLSI) and European Committee

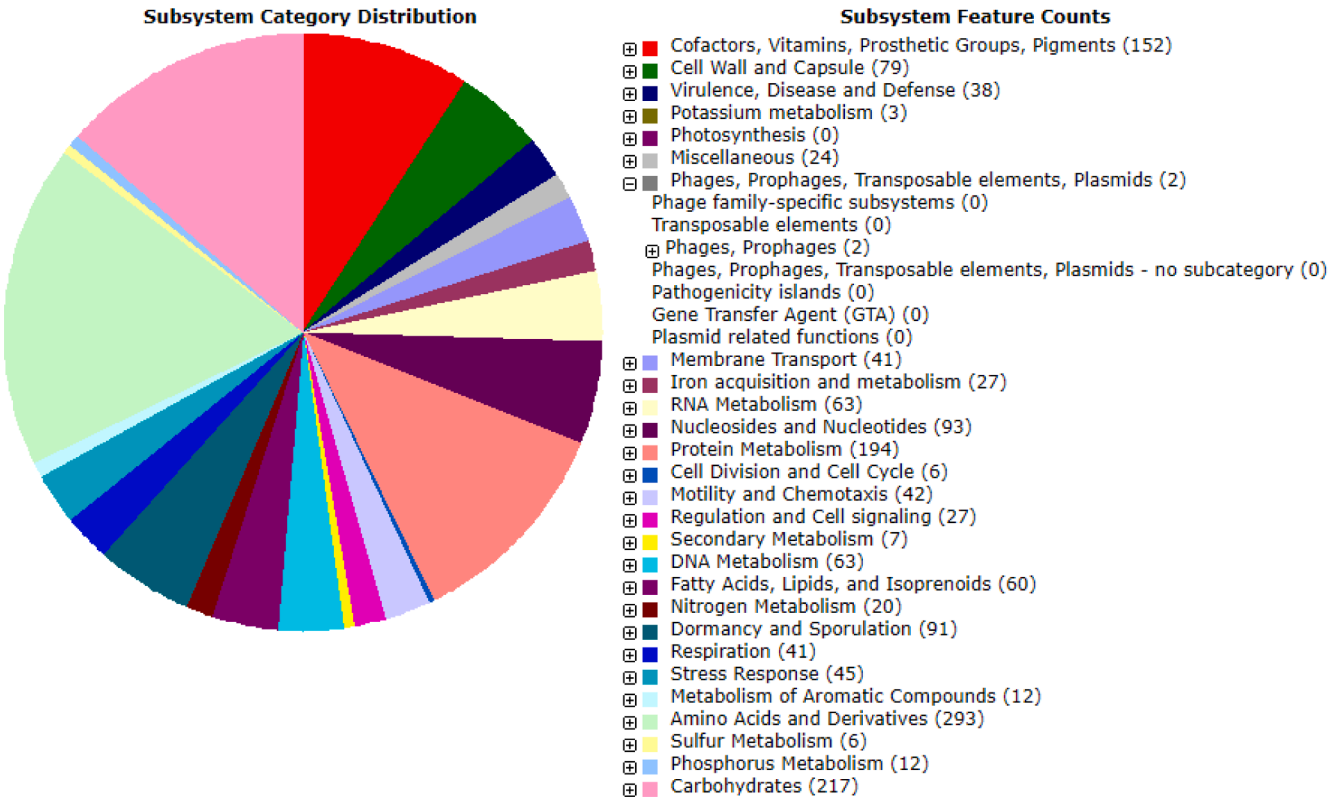

Fig. 4. Subsystem category distribution of *B. velezensis* HU-91 genome. Numeric values in brackets represent number of genes.

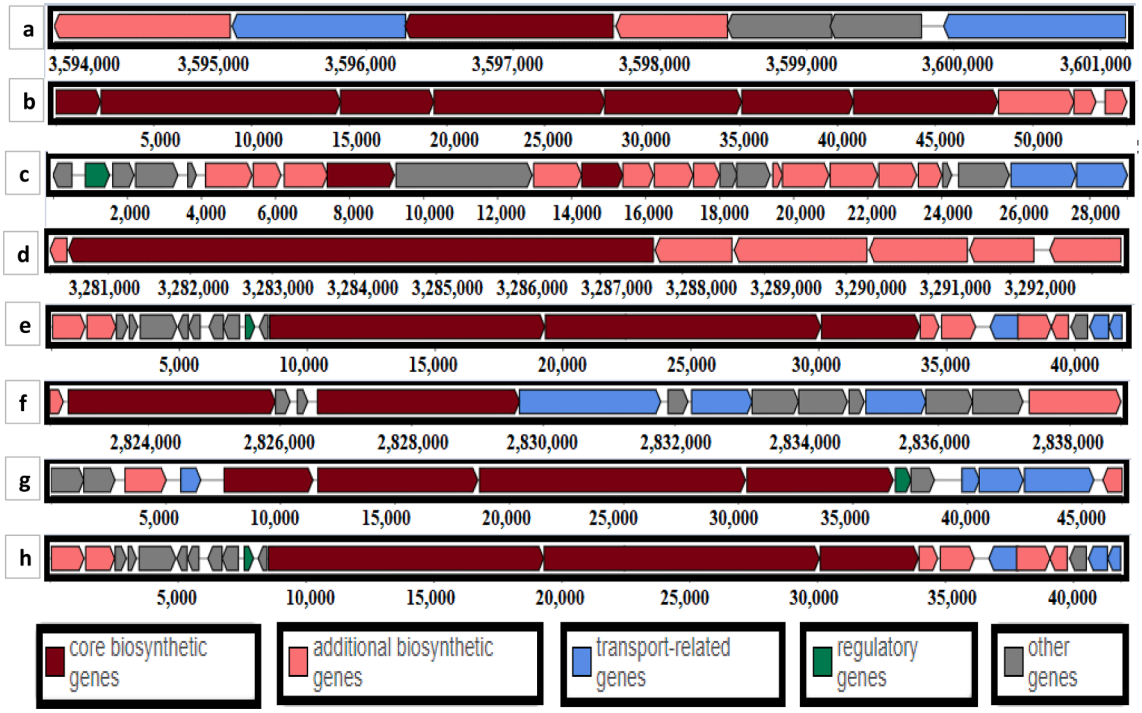

Fig. 5. Secondary metabolite gene clusters encoding antibiotics. a) Bacilysin b) Macrolactin, c) Bacillibactin d) Surfactin, and e) Amyloliquecifidin f) Locillomycin g) Difficidine h) Bacillaene. Numeric values show the position of genes.

on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Wash et al., 2022). Briefly, the antimicrobial discs were placed on the lawn of strain on agar plates and incubated overnight at 37°C. The inhibition zone was measured, and the strain was categorized as MDR, XDR or PDR. Multiple antimicrobial resistance index (MAR) was calculated by using the following formula (Dela Peña et al., 2022).

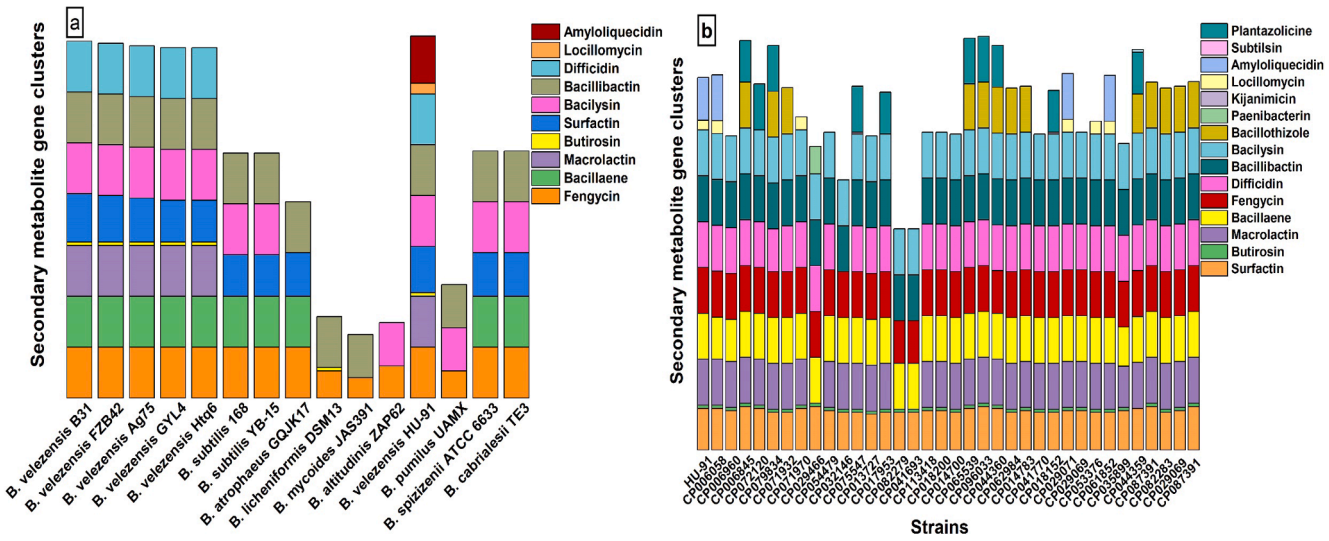

Fig. 6. Comparison of antibiotics biosynthetic gene clusters of *B. velezensis* HU-91. a) Comparison among different taxonomic groups (*Bacillus* spp.) b) Comparison among same taxonomic group (*B. amyloliquefaciens*).

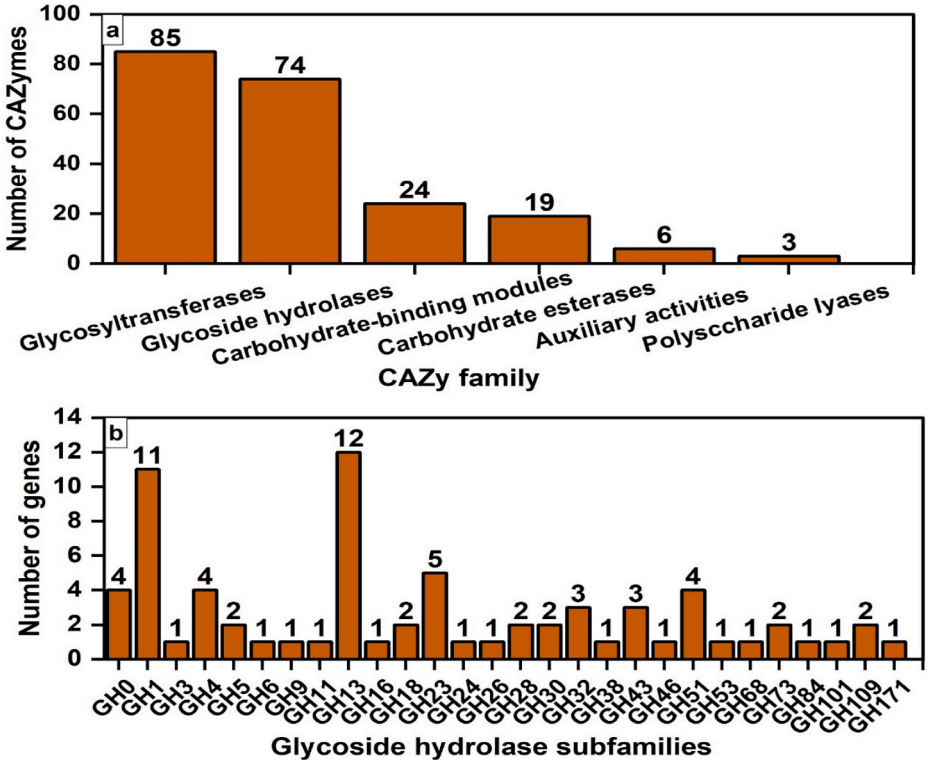

Fig. 7. Distribution of CAZymes in the genome of *B. velezensis* HU-91. a) Number of CAZymes families in HU-91b) Number of genes present in subfamilies of glycoside hydrolase involved in biosynthesis of major hydrolytic enzymes.

$$\text{MAR index} = a/b\ (a = \text{number of antimicrobial agents against which HU} - 91 \text{ shows resistance; } b = \text{total number of antimicrobial agents tested}).$$

2.6. Whole genome sequencing and analysis

Genomic DNA was isolated from the fresh bacterial culture by DNA extraction kit (Thermo Fisher Scientific, USA). Quantification was done by Nanodrop spectrophotometer. The genome of bacteria was sequenced commercially by Azenta life sciences USA C/O Alpha Genomics (Pvt) Ltd, Islamabad, Pakistan. Briefly, sequencing was performed using Illumina HiSeq 4000 platform. The quality of raw reads were filtered by FastQC version 0.11.9 (Chandra et al., 2021). De novo assembly of the FastQC filtered genome was done by Velevet assembler version 1.2.10 using *Bacillus velezensis* FZB42 as a reference strain.

2.6.1. Gene prediction and annotation

The genome was annotated using PROKA which is used for the rapid annotation of genome using Circular Genome Viewer (CG viewer). The comparison of the sequences of genome with neighbor strain was also uploaded by this tool. The protein coding genes were predicted by Rapid

Table 3
Biochemical and genetic profile of antimicrobial resistance in *B. velezensis* HU-91.

Antibiotic class	Resistance phenotype	MAR index	Resistance genotype
β lactams	ATM, FEP, AMP, AMX, OXA PEN	0.75	*Bcl, penP, bla2, blm, ccrA, blaB*
Fosfomycins	–	–	*FosB*
Glycopeptides	–	–	*vanT*
Lincosamides Streptogramins Oxazolidinones Phenicols Pleuromutilins	CLI	1	*ClbA, ImrB*
Macrolides	RIF	0.25	*msr, vmIR*
Tetracyclines	–	–	*tetA*
Fluoroquinolones	–	–	*gyrA-B*

ATM (Aztreonam), FEP (Cefepime), AMP (Ampicillin), AMX (Amoxicillin), OXA (Oxacillin), PEN (Penicillin), CLI (Clindamycin), RIF (Rifampin), MAR (multiple antimicrobial resistance), "-" (no resistance phenotype).

Annotation Subsystem Technology (RAST) which annotates the genome built upon SEED. This system identified protein-encoding, rRNA and tRNA genes, assigned specific functions to each gene and categorized them into subsystems. The genome was constructed in the form of a circular map by using CG viewer tool using default parameters (Narayanasamy et al., 2023).

2.6.2. Taxonomic classification of HU-91

The average nucleotide identity (ANI) was used for the phylogenetic analysis and similarity of nucleotides of HU-91 was calculated using OAT database (orthoANI) (Han et al., 2016).

2.6.3. Genome mining to elucidate the genetics of antagonism

The non-ribosomal peptides and antibiotics were predicted by antiSMASH 3.0 and compared with that of 15 closest strains belonging to same taxonomic group. The carbohydrate related enzymes involved in antifungal activities were detected by using dbCAN3 sever with a combination of DIAMOND and HMMER databases (Xu et al., 2022). The percent identity obtained from biosynthetic gene cluster was plotted as heatmap by using Originpro (version 2021).

2.6.4. Genome mining to elucidate the genetics of antimicrobial resistance

Antimicrobial resistance genes in the assembled genome were detected through comprehensive antibiotic resistance gene database (CARD) and homolog detection was done through loose, strict, and perfect algorithm.

2.6.5. Detection of CRISPR and plasmid

The genome of HU-91 was analyzed for the detection of CRISPR Cas system and plasmid by using CRISPR Cas Finder and Plasmid Finder on default settings (Kamruzzaman & Iredell, 2020). All the tools used for genome analysis were used in default settings.

3. Results

3.1. Antagonistic bacteria associated with wheat

Ten out of 50 bacterial isolates showed antifungal activity against one or more phytopathogens of wheat such as *F. graminearum*, *F. moniliforme*, *F. oxysporum* and *F. solani*. The antagonistic bacteria were found to be rhizospheric (6), root endophytic (2) and both rhizospheric as well as root endophytic (2) (Table 1). They had a variable colony morphology such as round, oval with irregular and smooth margins. Most of the isolates (n = 7) were Gram-positive while a few (n = 3) were Gram-negative (Table 1). The antagonistic bacteria showed antifungal activity against more than one phytopathogen (Table 1). The maximum number of strains antagonized *F. moniliforme,* followed by *F. oxysporum*, *F. graminearum* and *F. solani*. HU-91 showing maximum antagonistic activity was selected for further analysis.

3.2. Screening of HU-91 for lipopeptides production

HU-91 showed maximum adherence to hydrocarbons (80 %) which is close to the positive control *Bacillus cabrialesii* SM 93 (87 %) while the oil spreading, and emulsification activity of HU-91 was 2.1 cm and 73 % respectively (Table 2).

3.3. Antimicrobial resistivity of b. Velezensis HU-91

HU-91 exhibited a varied pattern of resistance against different antimicrobial classes. HU-91 exhibited highest resistance to β lactams and least resistance was shown against lincosamides, and macrolides (Fig. 1). HU-91 showed resistance to rifampin (RIF, 5 μg), clindamycin (CLI, 2 μg), aztronam (AZM, 30 μg), cefepime (FEP, 30 μg), ampicillin (AMP, 10 μg), amoxicillin (AML, 30 μg), oxacillin (OXA, 5 μg) and penicillin (PEN, 10 μg) (Table 2). The tested strain showed intermediate resistance against piperacillin (PIP, 100 μg). HU-91 exhibited susceptibility to all the antibiotics belonging to class nitrofurantoins, phenicols, glycopeptides, fluoroquinolones, aminoglycosides, oxazolidinones, tetracyclines and sulfonamides. HU-91 was categorized as multi drug resistance. The cumulative MAR index of HU-91 was higher than 0.2 while the MAR index based on different classes was variable i.e. 0.25 for macrolides, 1 for lincosamide, 0.7 for β lactams and 0 for nitrofurantoins, phenicol, glycopeptides, fluoroquinolones, aminoglycosides, oxazolidinones, tetracyclines and sulfonamides.

3.4. Whole genome analysis of b. Velezensis HU-91

The genome of HU-91 was assembled in 76 contigs (3,952,697 bp) with a mean GC content of 46.3 %. The total number of genes predicted was 3912, out of which, 3729 were protein-coding genes (118 total RNAs, 84 tRNA, 29 rRNA, and 5 nc RNA) (Fig. 2). BLAST similarity search indicates sequence similarity with *Bacillus velezensis*. The genome sequence of HU-91 is available at the Gene Bank under accession number JAAHBR000000000.1. The genome was classified into 27 subsystems with genes encoding different functions (Fig. 4).

3.5. Phylogenomic analysis

Pairwise genome comparison of HU-91 showed sequence similarity with *Bacillus velezensis* FZB42 based on the phylogenetic analysis of whole genome with average nucleotide identity of 98.49 (Fig. 3).

3.6. Antifungal metabolites detected in genome of b. Velezensis HU-91

A total of ten secondary metabolites genes cluster comprising six non ribosomal peptide synthesis (NRPS), one for each metabolite (bacillibactin, locillomycin, fengycin, bacillaene) and two NRPS for surfactin biosynthesis was identified (Fig. 5). The genome of HU-91 showed similarity in their biosynthetic gene cluster with its closely related strains such as *B. velezensis*, *B. amyloliquefaciens* and *B. subtilis* (Fig. 6a). HU-91 showed variation in its biosynthetic gene clusters with that of

distinctly related genera (Fig. 6b). The genome of HU-91 carried a unique metabolite locillomycin, comprising 21 % identity with *B. subtilis* and butirosin, comprising 7 % identity with *B. circulans*.

3.7. Analysis of CAZymes in genome of HU-91

A total of 205 CAZymes were detected in the whole genome of HU-91. The CAZymes (205) were classified into 5 families such as glucoside hydrolases (68), glycosyltransferases (85), carbohydrate esterases (19), carbohydrate binding modules (24), auxiliary activities (6) and polysaccharide lyases (3). The genes belonging to glucoside hydrolase family were involved in production of enzymes such as chitinases, pectinases, and beta glucanases respectively (Fig. 7).

3.8. Detection of antimicrobial resistance genes in b. Velezensis HU-91

The *B. velezensis* HU-91 showed genes responsible for the antimicrobial resistance (Table 3). The antimicrobial resistance genes identified belonged to different antimicrobial classes. Furthermore, a CRISPR Cas system (136 bp) was identified while no plasmid was detected in this strain (Fig. 4).

4. Discussion

Rhizobacteria are an important part of soil ecosystem playing role in soil fertility, nutrient recycling, and control phytopathogens. The use of rhizobacteria as biocontrol agent is an ecofriendly strategy. However, many rhizobacteria species isolated from soil have antimicrobial resistance genes (Zhang et al., 2021). In the current study, rhizobacteria were isolated from rhizosphere and endosphere of wheat which was grown in monocropping system under semi-arid region. In mono cropped wheat, the rhizospheric bacteria were higher in number than that of the endophytic bacteria. This difference may be due to the highly nutritious environment of rhizosphere which makes it a hotspot for the microbial activity, habitat specificity, and passive bacterial transport through roots (Vandana et al., 2021).

These rhizobacteria showed variable antagonistic activity against more than one *Fusarium* pathogens. The antagonistic property of a bacteria depends upon the type of pathogen, their genetic makeup, production of secondary metabolites, and hydrolytic enzymes. The antagonistic activity of rhizobacteria could also be due to the repeated mono culturing of wheat which governs the rhizospheric microbial community and determines the nature of soil like suppressive or non-suppressive (Ullah et al., 2020).

HU-91 showed maximum antagonistic activity against *Fusarium* spp. A tremendous amount of literature has been documented on the antagonistic behaviors of *B. velezensis* against *Fusarium* (Diabankana et al., 2022; Moreno-Velandia et al., 2021). The strain also produces lipopeptides which are broad spectrum antifungal metabolites.

Biocontrol agents could be a carrier of antimicrobial resistance genes. Therefore, HU-91 was screened for drug resistance traits. In the current study, HU-91 was categorized as MDR because it resisted three antimicrobial classes. The highest resistance was shown against β lactam while lowest was shown against macrolides, and lincosamides. The MAR index of HU-91 was higher than 0.2 which exhibited a potential risk associated with this beneficial bacterium. The resistance/susceptibility of bacteria to these antibiotics have been reported previously by Fraccalvieri et al., (2022). The resistance/susceptibility of bacteria to antimicrobial agents depends upon several factors such as continuous exposure to antimicrobial contaminants, production of antibiotic degrading enzymes by bacteria and presence of efflux pumps (Bianco et al., 2021). The bio control agents could use the same mechanisms of resistance for the antibiotics production and resistance (multi drug efflux pumps) (Zhang et al., 2021).

These traits of HU-91 were further validated by the whole genome analysis. The genomic sequencing of HU-91 revealed 67 contigs and it was identified as a member of *Bacillus velezensis*. The size of the HU-91 genome was somewhat comparable with its closest strain *Bacillus velezensis* FZB42 (3918596 bp) as reported earlier (Zaid et al., 2022). The inclusion of some genes involved in other unique functions may account for a slight variation in genome size. The phylogenetic analysis based on the whole genome revealed the association of HU-91 with *B. velezensis* FZB42. ANI a new metric for pairwise genome comparison also confirmed the 98 % similarity of HU-91 with *Bacillus velezensis* (Han et al., 2016).

WGS predicted ten secondary metabolites genes clusters in HU-91 genome. The presence of antimicrobial functions such as surfactin, fengycin, bacilleane, difficidin, macrolactin and bacillibactin (catechol iron siderophore) endows the potential to HU-91 as effective biocontrol agent (Nifakos et al., 2021; Zhang et al., 2022). Biosynthetic gene clusters for butirosin, locillomycin and amyloliquecidin showed 7 %, 21 % and 93 % homology which showed a sequence similarity with *B. amyloliquefecians* genome (Samaras et al., 2021). Genome mining also revealed the presence of carbohydrate active enzymes which cleave the polysaccharides in fungal cell wall into simple monomers (Tsalgatidou et al., 2022). CAZymes analysis of HU-91 showed genes involved in the degradation of fungal cell wall such as chitinases and β glucanase. The presence of these enzymes serves as the inhibitory action of HU-91 which indicates that this strain could play an important role in suppressing the phytopathogens.

The annotated genome carried antimicrobial resistance genes by following the default settings in CARD database. HU-91 genome carried a CRISPR Cas system. CRISPR Cas system plays an important role in bacterial immunity and protects the bacterial genome from invading plasmids (Tao et al., 2022). The presence of CRISPR Cas system could be a possible reason for the absence of plasmid in HU-91 genome. These findings are in line with previous studies (Wheatley & MacLean, 2021). However, the presence of antimicrobial resistant genes in chromosomal DNA could lead to the accumulation of AMRs in the soil upon repeated inoculation. Hence the presence of PGP traits, antibiotic resistance genes, and antagonism provide a new insight into the adaptation of this strain as a potential new candidate for plant growth promotion, antagonism, and drug resistance.

5. Conclusion

The present study depicts that *B. velezensis* HU-91 harbors antifungal and antimicrobial resistance traits but the presence of CRISPR Cas system and absence of plasmid make it potentially safe. However, it is necessary to screen these biocontrol agents before applying them on a commercial scale.

Consent to participate

All authors consent to participate in the manuscript publication.

Consent for publication

All authors approved the manuscript to be published.

Availability of data and material

The data supporting the conclusions of this article are included within the article.

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Author contributions

PW and MNH have contributed in conceptualization, acquisition of data, and drafting. HU and HY have helped in sampling, research co-ordination, editing and analysis of data. WH, NK, MNH and AA have contributed in the analysis and interpretation of data, revising and editing the manuscript, and acquisition of funding.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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